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(54) **COMPOSITION COMPRISING HISTONE DEACETYLASE INHIBITOR AND ESTROGEN RECEPTOR ANTAGONIST FOR USE IN A METHOD OF TREATMENT OF CANCER**

ZUSAMMENSETZUNG MIT HISTON-DEACETYLASE-INHIBITOR UND ÖSTROGENREZEPTOR-ANTAGONISTEN, ZUR VERWENDUNG IN EINEM VERFAHREN ZUR BEHANDLUNG VON KREBS

COMPOSITION COMPRENANT UN INHIBITEUR D'HISTONE-DÉSACÉTYLASE ET UN ANTAGONISTE DU RÉCEPTEUR D'OESTROGÈNE POUR UTILISATION DANS UNE MÉTHODE DE TRAITEMENT DU CANCER

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- **NOEMI ARRIGHETTI ET AL: "Drug Combinations with HDAC Inhibitors in Antitumor Therapy", CRITICAL REVIEWS IN ONCOGENESIS, vol. 20, no. 1-2, 1 January 2015 (2015-01-01), pages 83 - 117, XP055369440, ISSN: 0893-9675, DOI: 10.1615/CritRevOncog.2014012378**

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Description

FIELD OF THE INVENTION

[0001] The present invention relates to the field of pharmaceuticals. More specifically, the invention pertains to a novel composition for use in a method of treatment of gliomas.

BACKGROUND OF THE INVENTION

[0002] Cancer is an abnormal growth of cells and there are several types of cancer, including breast cancer, skin cancer, lung cancer, colon cancer, prostate cancer, brain cancer and lymphoma. Brain tumors are abnormal growth of cells in the brain. High grade gliomas (HGG) or malignant glioma is a deadly form of human cancer. 'Cancer stem cells' (CSC) within solid tumors that compel tumor formation and growth are reported to confer resistance to conventional chemotherapy and radiotherapy. The prognosis for patients with high-grade gliomas (GBM-glioblastomas) is generally poor (in patients with IDH mutations which is a feature in younger patients, the survival is poor). Despite combined modality treatment including surgical resection and radiation, a minority of patients live beyond 2 years. However, various chemotherapeutic agents used as combination therapy targeting multiple pathways are becoming a fast growing area of research.

[0003] Selective estrogen receptor modulators (SERM) used as anti-tumoral agents include raloxifene, tamoxifen or toremifine. Tamoxifen ((Z)-2[p[(1,2-diphenyl-1-butenyl)phenoxyl]-N,N-dimethyl amine citrate) is a potent estrogen receptor (ER) antagonist that has been extensively used to treat ER-positive breast cancer. Tamoxifen is a selective estrogen receptor modulator (SERM, indicated to treat women diagnosed with hormone-receptor-positive, early-stage breast cancer after surgery to reduce the risk of recurrence. Tamoxifen also is used to reduce breast cancer risk in women who haven't been diagnosed but are at higher-than-average risk for the disease. However, tamoxifen is not indicated in hormone-receptor-negative breast cancer.

[0004] Histone deacetylase inhibitors (HDACi) like valproic acid, sodium butyrate, vorinostat, romidepsinor and trichostatin A (TSA) are known to be effective anticancer agents through epigenetic modulation. Valproic acid (Valproate; VPA) or valpromide (VPM) or sodium valproate or valproate semisodium forms are medications primarily used to treat epilepsy and prevention of seizures.

[0005] Prostate apoptosis response-4 (Par-4) is a naturally occurring tumor suppressor protein that is capable of inducing apoptosis. Since membrane GRP78 is overexpressed in most cancer cells but not normal cells, extracellular or secretory Par-4 induces apoptosis by binding to membrane GRP78. Though secretory Par-4 induces apoptosis in cancer cells, its potential in drug-resistant tumors remains to be fully explored.

[0006] Certain prior arts disclose certain combination of drugs used for treating cancer. For instance, US 20090048156A1 discloses a method for treating hormone resistant breast cancer comprising administration of HDAC inhibitors and hormone targeted drugs. However, US'156 fails to disclose the use of such combination for treating malignant gliomas by targeting Par-4-GRP78 pathway.

[0007] US 20080085874A1 discloses method of treating estrogen receptor positive breast cancer comprising administration of combination comprising HDAC inhibitor in combination with hormonal therapy wherein the HDAC inhibitor is not valproic acid. However, US'874 fails to disclose the combination of tamoxifen and valproic acids for treating malignant gliomas.

[0008] Publication P N Munster et al, "A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer", British Journal Of Cancer, London, (20110510), vol. 104, no. 12, pages 1828 - 1835, is drawn to inferences of phase II clinical trials and concludes that the combination of histone deacetylase inhibitor vorinostat and estrogen receptor (ER) antagonist tamoxifen is well tolerated and exhibits encouraging activity in reversing hormone resistance . The publication aims to provide a solution to hormone therapy-resistant breast cancer by suggesting use of HDAC inhibitors (exemplified as vorinostat) could re-sensitise tamoxifen-resistant cells to hormone therapy.

[0009] Publication WO2009142968 discloses valproic acid (VPA) and its salts in combination with hormonal therapy, including antiestrogens, aromatase inhibitors, and selective estrogen receptor modulators, for treatment of cancer, such as human ERa -positive breast cancer cells. However nowhere mentions the activity of the disclosed combination against glioma or malignant gliomas and only provides a combination for activity against the treatment and prevention of breast cancer.

[0010] Publication WO2008027837 discloses valproic acid (VPA), carbamazepine, and other HDAC inhibitors in combination with hormonal therapy, including antiestrogens and aromatase inhibitors, on human ERα-positive breast cancer cells, however nowhere mentions the activity of the disclosed combination against glioma or malignant gliomas and only provides a combination for activity against the treatment and prevention of breast cancer.

[0011] The publication of Noemi Arrighetti et al. "Drug Combinations with HDAC Inhibitors in Antitumor Therapy" Critical Reviews In Oncogenesis, vol.20, no. 1-2, 1 January 2015, pages 83-227, studies paradoxical role of HDACs as tumor suppressors, the treatment of tumor cells with HDAC inhibitors (HDACi) that induces a range of effects including apoptosis, cell cycle arrest, differentiation and senescence, modulation of immune response, and altered angiogenesis. The composition comprising histone deacetylase inhibitor (HDACi) and an estrogen receptor (ER) antagonist is not disclose

there, nor the specific dose range of HDACi is disclosed in detailed. Noemi Arrighetti et al. discloses glioma treatment with valproate however is completely silent of combination of valproate and tamoxifene for the treatment of glioma. There is also no synergy between those two compounds disclosed in the treatment of glioma.

[0012] The above cited prior arts disclose combinations of HDAC inhibitor with hormone targeted drugs in breast cancer. There remains a need for effective novel combination of HDAC inhibitor and hormone targeted drugs having anticancer activity against drug resistant malignant gliomas. Therefore, there is a need to develop new strategies for controlling the cell growth and killing of cancerous cells in high grade gliomas or malignant gliomas.

OBJECT OF THE INVENTION

[0013] An object of the invention is to provide a novel, synergistic composition of HDAC inhibitor and estrogen receptor (ER) antagonist having anticancer effects by targeting Par4/GRP78 pathway.

SUMMARY OF THE INVENTION

[0014] The present invention is based on a phamaceutical synergistic composition comprising histone deacetylase inhibitors (HDACi) selected from valproic acid, sodium valproate, valproate semisodium, valpromide, in combination with estrogen receptor (ER) antagonist which is tamoxifen for use in a method of treating gliomas.

[0015] The scope of the present invention is defined in the claims; any other disclosure in the present description not protected by the appended claims, regardless of whether it is indicated as being disclosed, preferred or exemplified, is provided for information purposes, only

[0016] Further disclosed histone deacetylase inhibitors (HDACi) are from the group comprising valproic acid, sodium butyrate, vorinostat, romidepsinor and trichostatin A (TSA). The disclosed estrogen receptor (ER) antagonist are from the group comprising tamoxifen, raloxifene, fulvestrant, and torimefene.

[0017] The compositions of the invention are for use in a method of treating gliomas, in particular high grade gliomas or malignant gliomas.

BRIEF DESCRIPTION OF FIGURES

[0018]

Fig. 1 depicts cell viability assessed by MTT assay in GBM cell lines- LN-18 and LN-229 using tamoxifen (TAM) at concentration of 5 μg/ml, 10 μg/ml and 15 μg/ml; individually and in combination with Valproic Acid (VPA) (2 mM) wherein the cells were cultured as monolayer (ML) or Multicellular Spheroids (MCS). Unt-open bar- represents untreated cells and hatched bar -VPM (2mM). Further, the open bars (in 5, 10 15) depict TAM alone and the hatched bars depict TAM in combination with VPA. Fig 1 also depicts the P values (represented as *) significant for differences between TAM alone versus TAM+VPA. The images are LN-18 cells grown as 2D cultures- ML and 3D-MCS.

Fig. 2 depicts the effect of Valproic acid (VPA) on tamoxifen (TAM) -induced apoptosis in LN-18 cells cultured as monolayer (ML) or Multicellular Spheroids (MCS). The cells cultured as monolayers were treated with tamoxifen (10μg/ml) or valproic acid (2mM) alone or in combination for 24 hrs (upper panel), and the LN-229 cells cultured as multicellular spheroids were treated with tamoxifen (10μg/ml) or valproic acid (2mM) alone or in combination for 48 hrs (Lower panel). The P values (represented as *) are significant for differences between TAM alone versus TAM+VPA.

Fig. 3 depicts the effect of Valpromide (VPM), an analogue of VPA causing sensitization of tamoxifen (TAM) which induced cell death in LN-18 cells cultured as monolayer (ML). The figure depicts that VPA and VPM show similar responses in combination with TAM in reducing cell viability in LN-18 cells. The P values (represented as *) are significant for differences between TAM alone versus TAM+VPA/VPM.

Fig. 4 depicts the effect of combination of tamoxifen (10μg/ml) and valproic acid (2mM) on the levels of secretory Par-4 and expression of GRP78 in LN-229 cells wherein the control (C) represents untreated cells, V represents for valproic acid, T represents for tamoxifen and V+T represents combination of tamoxifen and valproic acid. The LN-18 cells were treated with tamoxifen or valproic acid alone or in combination for 48 hrs.

Fig. 5 depicts the effect of tamoxifen (12 μg/ml) or valproic acid (2mM) alone or combination of tamoxifen and valproic acid on Par-4 expression in LN-229 cells for 24 hr. GAPDH was used as loading control and control group (C) represents untreated cells.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The present invention is based on synergistic composition comprising histone deacetylase inhibitors (HDACi) selected from valproic acid, sodium valproate, valproate semisodium, valpromide in combination with a hormone-targeted drug i.e. estrogen receptor (ER) antagonist which is tamoxifen for use in the treatment of cancer selected from gliomas.

[0020] The invention relates to a composition comprising histone deacetylase inhibitor (HDACi) and an estrogen receptor (ER) antagonist for use in a method of treatment of cancer, wherein the cancer is selected from glioma, malignant glioma, high grade glioma, and wherein histone deacetylase inhibitor (HDACi) is selected from valproic acid, sodium valproate, valproate semisodium,

valpromide, and wherein estrogen receptor (ER) antagonist is tamoxifen.

[0021] In the preferred pharmaceutical composition for use the histone deacetylase inhibitor (HDACi) is the valproic acid in the range of 0.5 mM to 5 mM, more preferably at 1mM to 5 mM and most preferably at 1 to 3 mM.

[0022] In the preferred pharmaceutical composition for use the tamoxifen is in the range of 1 μg/ml to 20 μg/ml, more preferably at 5 μg/ml to 15 μg/ml..

[0023] Preferably the pharmaceutical composition for use shows the effect in RTK/Ras/PI3K signaling pathway, pRB signaling pathway, PI3K/AKT/mTOR pathway, p53 pathway, Par-4/GRP78, preferably in Par-4/ GRP78 pathway.

[0024] The pharmaceutical composition for use preferably , further comprises excipients selected from the group comprising preservatives, buffering agents, salts, carriers, diluents.

[0025] The present invention is capable of being administered to a subject. A "Subject" herein is any mammal, preferably the subject is a human. The subject includes within its scope patients or any diseased mammal or human.

[0026] The present invention is based on combined synergistic combination effect of histone deacetylase inhibitor (HDACi) wherein histone deacetylase inhibitor (HDACi) is selected from valproic acid, sodium valproate, valproate semisodium, valpromide with estrogen receptor (ER) antagonist which is tamoxifen. Without being limited to the theory, the novel composition for use of the present invention induces its anticancer effects through intervention of signal transduction pathway in gliomas. It hence envisages that this composition for use will also be effective in other pathways, preferably in Par-4/ GRP78 pathway.

[0027] The synergistic composition for use may also affect other signal transduction pathways such as RTK/Ras/PI3K signaling pathway, pRB signaling pathway, PI3K/AKT/mTOR pathway, p53 pathway, Par-4/GRP78 pathway individually or in combination thereof as promising targets for killing of cancer cells.

[0028] The histone deacetylase inhibitors (HDACi) may be selected from the group comprising valproic acid, sodium valproate, valproate semisodium, valpromide, sodium butyrate, vorinostat, romidepsinor and trichostatin A (TSA), preferably valproic acid.

[0029] The concentration of valproic acid/valpromide may be used in the range of 0.5mM to SmM, more preferably at 1mM to 5mM and most preferably at 1-3 mM.

[0030] The estrogen receptor (ER) antagonist may be selected from the group comprising tamoxifen, raloxifene, fulvestrant, and torimefene, preferably tamoxifen.

[0031] The concentration of tamoxifen may be used in the range of 1 μg/ml to 20 μg/ml, more preferably at 5 μg/ml to 15 μg/ml.

[0032] Surprisingly, it has been found that the composition of tamoxifen and valproic acid is synergistic and induce apoptosis in glioma cells in a dose-dependent manner that has been demonstrated *in vitro* on various brain tumor cell lines such as LN-229, T98G [T98-G], LN-18, M059K, M059J, U-87 MG, U-118 MG, or a composition thereof.

[0033] Additional ingredients may be included with the composition for use of the present invention having anticancer effect on gliomas. Such ingredients may be any actives that may be used for treatment of cancer.

[0034] In reference embodiment, the present invention discloses a composition comprising the combination of histone deacetylase inhibitor (HDACi) and estrogen receptor (ER) antagonist for use in a method of treatment of cancer, along with pharmaceutically acceptable excipients selected from the group comprising preservatives, buffering agents, salts, carriers, diluents. Such additional ingredients include other active agents, preservatives, buffering agents, salts, carriers, diluents, or other pharmaceutically acceptable ingredients. As mentioned VPM also works though it is not a HDAC inhibitor. The finding suggests that the anti-cancer effect is independent of its activity as HDAC inhibitor.

[0035] There is disclosed a composition comprising the combination, for treating cancer, drug-resistant tumors, cancer stem cells, malignant gliomas or high-grade gliomas by administering a pharmaceutically effective amount to a subject.

[0036] The synergistic combination comprising histone deacetylase inhibitor (HDACi), preferably valproic acid and estrogen receptor (ER) antagonist, preferably tamoxifen may induce the expression of intrinsic and secretory pro-apoptotic protein Par-4 which further interacts with the cell surface receptor GRP78 (glucose-regulated protein 78) to induce cancer cell apoptosis in a specific manner.

[0037] The described combination is used for treatment of cancer, drug-resistant tumors, cancer stem cells, malignant gliomas or high-grade gliomas.

[0038] There is described a composition comprising the combination of histone deacetylase inhibitors (HDACi) and an estrogen receptor (ER) antagonist, for treating cancer, drug-resistant tumors, cancer stem cells, malignant gliomas or high-grade gliomas by administering a pharmaceutically effective amount to a subject or for its effect in RTK/Ras/PI3K signaling pathway, pRB signaling pathway, PI3K/AKT/mTOR pathway, p53 pathway, Par-4/GRP78, preferably in Par-4/ GRP78 pathway.

[0039] The histone deacetylase inhibitors (HDACi) is selected from the group comprising valproic acid, sodium valproate, valproate semisodium, valpromide, sodium butyrate, vorinostat, romidepsinor and trichostatin A (TSA), preferably valproic acid and is present in the range of 0.5mM to SmM, more preferably at 1mM to 5mM and most preferably at 1 to 3 mM.

[0040] The estrogen receptor (ER) antagonist is selected from the group comprising tamoxifen, raloxifene, fulvestrant, and torimefene, preferably tamoxifen and is

in the range of 1 μg/ml to 20 μg/ml, more preferably at 5 μg/ml to 15 μg/ml and most preferably at 5 μg/ml to 15 μg/ml.

[0041] There is also described a method of intervening in RTK/Ras/PI3K signaling pathway, pRB signaling pathway, PI3K/AKT/mTOR pathway, p53 pathway, Par-4/GRP78, preferably in Par-4/ GRP78 pathway by administration of the disclosed combination.

ADVANTAGES

[0042]

1. The synergistic composition of the present invention having anticancer effects is effective against malignant gliomas or high-grade gliomas.
2. The synergistic composition of the present invention may effectively act against drug-resistant tumors or cancer stem cells.
3. The composition of tamoxifen and valproic acid may induce apoptosis in cancer cells by targeting specific Par-4/GRP78 pathway, which is unexpected.

EXAMPLES

[0043] The following examples are given by the way of illustration of the present invention and therefore should not be construed to limit the scope of the present invention.

Example 1: Effect of tamoxifen (TAM) and VPA in LN-18 cells and LN229 cells

[0044] MTT assay was employed for assessing the combined action of tamoxifen (TAM) and VPA. LN-18 cells and LN-229 were seeded in 96 well plate (5000 cells/100ul/well) in complete medium and cultured for 24 hr in two culture models-monolayer and 3D culture. Cells were treated with tamoxifen (TAM) at 3 concentrations-5μg/ml, 10μg/ml and 15μg/ml either individually or in composition with Valproic Acid (VPA) (2 mM) for 24 hr. MTT was added and after 4 hr the crystals were solubilized using DMSO. Absorbance was recorded at 490 nm (test filter) and 570 nm (reference filter). The % cell viability in treated cells was calculated considering the readings in untreated cells as 100%. The results are presented at Fig.1. From Fig.1 it is evident that the effect of TAM was dose dependent in monolayer, TAM was toxic at 15μg/ml in LN-18 cells. More importantly it can be seen that VPA enhanced the effect of TAM (10 μg/ml) significantly in LN-18 cells cultured as monolayer culture and that MCS of LN-18 cells were resistant to TAM treatment. Furthermore, VPA enhanced the effect of TAM at 10μg/ml in ML and at 15 μg/ml in MCS of LN-18 cells. MCS of LN 229 cells were resistant to TAM treatment and VPA enhanced the effect significantly in cells treated with TAM at 15 μg/ml.

[0045] From Fig.1 it is evident that the composition of the present invention is synergistic.

Example 2: Effect of VPA on induction of apoptosis with of tamoxifen (TAM) in LN-18 cells

[0046] LN-18 cells were seeded in 6 well plate (0.25 million cells/well) in complete medium and grown as 2D (monolayer) or 3D (MCS) cultures. Cells were treated with tamoxifen (TAM) -10 μg/ml either individually or in combination with Valproic Acid (VPA) (2 mM). Monolayer were treated for 24 hr and MCS were treated for 48 hr. Cells were fixed and stained with propidium Iodide and analysed by flowcytometry for cell cycle analysis. Data was acquired for 20,000 cells on linear scale (FL2A) and the cell population in pre-G0/G1 phase representing the apoptotic population was measured. The results are presented at Fig 2. From Fig 2., it can be seen that VPA enhanced the apopototic effect of TAM (10μg/ml) significantly in monolayer culture of LN-18 cells treated for 24 hr. VPA enhanced the apopototic effect of TAM (10μg/ml) significantly in MCS culture of LN-18 cells treated for 48 hr. In fig 2, the synergistic effect of the combination being the effect of apoptosis. Statistically significant difference is observed between apoptosis induced by TAM alone Vs. TAM + VPA is clearly seen.

Example 3: Comparison of VPA and VPM in combined action with tamoxifen (TAM)

[0047] LN-18 cells (ML) were seeded in 96 well plate (5000 cells/100ul/well) in complete medium and cultured for 24 hr. Cells were treated with tamoxifen (TAM) at 10 μg/ml either individually or in combination with Valproic Acid (VPA) (2 mM) or VPM (2mM) for 24 hr. MTT was added and after 4 hr the crystals were solubilized using DMSO. Absorbance was recorded at 490 nm (test filter) and 570 nm (reference filter). The % cell viability with treatment was calculated with readings in untreated cells considered as 100 %. The results are presented at Fig. 3. It can be seen from Figure 3 that there was no significant difference in the effect induced by VPA and VPM in combination with TAM (10μg/ml and 15μg/ml) on cell viability in LN-18 cells treated for 24 hr.

Example 4: Evaluation of expression of intracellular and secretory prostate apoptosis response (Par)-4 in LN-18 cells

[0048] LN-18 cells were seeded in 6 well plate (0.3 million cells/well) and treated with TAM (10μg/ml) and VPA (2mM) either individually or in combination for 48 hr.

[0049] Cell lysates were assessed for expression of intracellular prostate apoptosis response (Par)-4 and secretory Par-4 was measured in supernatants. Western blotting was done using 10% gels and the proteins were identified using specific antibodies followed by probing with luminol-based Enhanced Chemiluminescent (ECL)

western blotting substrate. The results are presented at Fig 4. From Fig 4, it can be seen that both cellular and secretory Par-4 was enhanced with TAM and a combination of TAM and VPA further enhanced the level indicating a synergistic effect.

Example 5: Evaluation of expression of intracellular prostate apoptosis response (Par)-4 in LN-229 cells

[0050] LN-229 cells were seeded in 6 well plate (0.3 million cells/well) and treated with TAM (12 μg/ml) and VPA (2mM) either individually or in combination for 24 hr. Cell lysates were assessed for expression of intracellular prostate apoptosis response (Par)-4. Western blotting was done using 10% gels and the proteins were identified using specific antibodies followed by probing with luminol-based Enhanced Chemiluminescent (ECL) western blotting substrate. The results are presented at Fig 5. From Fig 5, the VPA induced cellular Par-4 expression at 24 hr may be seen. The combined effect of TAM and VPA was higher compared to TAM alone clearly demonstrating synergy.

Claims

1. A pharmaceutical composition comprising histone deacetylase inhibitor (HDACi) and an estrogen receptor (ER) antagonist for use in a method of treatment of cancer,

   wherein the cancer is selected from glioma, malignant glioma, high grade glioma, and
   wherein histone deacetylase inhibitor (HDACi) is selected from valproic acid, sodium valproate, valproate semisodium, valpromide, and
   wherein estrogen receptor (ER) antagonist is tamoxifen.

2. The pharmaceutical composition for use according to claim 1, wherein the histone deacetylase inhibitor (HDACi) is the valproic acid.

3. The pharmaceutical composition for use according to claims 1-2, wherein the histone deacetylase inhibitors (HDACi) is in the range of 0.5 mM to 5 mM, more preferably at 1mM to 5 mM and most preferably at 1 to 3 mM.

4. The pharmaceutical composition for use according to claims 1-3, wherein the tamoxifen is in the range of 1 μg/ml to 20 μg/ml, more preferably at 5 μg/ml to 15 μg/ml.

5. The pharmaceutical composition for use according to claims 1-4, wherein it is for the effect in RTK/Ras/-PI3K signaling pathway, pRB signaling pathway, PI3K/AKT/mTOR pathway, p53 pathway, Par-4/GRP78, preferably in Par-4/ GRP78 pathway.

6. The pharmaceutical composition for use according to claims 1-5, wherein the composition further comprises excipients selected from the group of preservatives, buffering agents, salts, carriers, diluents.

Patentansprüche

1. Pharmazeutische Zusammensetzung, umfassend einen Histon-Deacetylase-Inhibitor (HDACi) und einen Östrogenrezeptor (ER)-Antagonisten zur Verwendung in einem Verfahren zur Behandlung von Krebs,

   wobei der Krebs ausgewählt ist aus Gliom, malignem Gliom, hochgradigem Gliom, und
   wobei der Histon-Deacetylase-Inhibitor (HDACi) ausgewählt ist aus Valproinsäure, Natriumvalproat, Valproat-Halbnatrium, Valpromid, und
   wobei der Östrogenrezeptor (ER)-Antagonist Tamoxifen ist.

2. Pharmazeutische Zusammensetzung zur Verwendung nach Anspruch 1, wobei der Histon-Deacetylase-Inhibitor (HDACi) Valproinsäure ist.

3. Pharmazeutische Zusammensetzung zur Verwendung nach den Ansprüchen 1-2, wobei der Histon-Deacetylase-Inhibitor (HDACi) im Bereich von 0,5 mM bis 5 mM, bevorzugter bei 1 mM bis 5 mM und am meisten bevorzugt bei 1 bis 3 mM liegt.

4. Pharmazeutische Zusammensetzung zur Verwendung nach den Ansprüchen 1-3, wobei das Tamoxifen im Bereich von 1 μg/ml bis 20 μg/ml, bevorzugter bei 5 μg/ml bis 15 μg/ml liegt.

5. Pharmazeutische Zusammensetzung zur Verwendung nach den Ansprüchen 1-4, wobei sie für die Wirkung auf den RTK/Ras/PI3K-Signalweg, den pRB-Signalweg, den PI3K/AKT/mTOR-Signalweg, den p53-Signalweg, den Par-4/GRP78-Signalweg, vorzugsweise auf den Par-4/GRP78-Signalweg, bestimmt ist.

6. Pharmazeutische Zusammensetzung zur Verwendung nach den Ansprüchen 1-5, wobei die Zusammensetzung ferner Hilfsstoffe umfasst, die aus der Gruppe von Konservierungsmitteln, Puffersubstanzen, Salzen, Trägern und Verdünnungsmitteln ausgewählt sind.

Revendications

1. Une composition pharmaceutique comprenant un

inhibiteur d'histone-désacétylase (HDACi) et un antagoniste du récepteur d'œstrogène (ER) à utiliser dans une méthode de traitement du cancer,

où le cancer est choisi parmi le gliome, le gliome malin, le gliome de haut grade et
où l'inhibiteur d'histone-désacétylase (HDACi) est choisi parmi l'acide valproïque, le valproate de sodium, le valproate semisodique, le valpromide, et
où l'antagoniste du récepteur d'œstrogène (ER) est le tamoxifène.

2. La composition pharmaceutique à utiliser selon la revendication 1, où l'inhibiteur d'histone-désacétylase (HDACi) est l'acide valproïque.

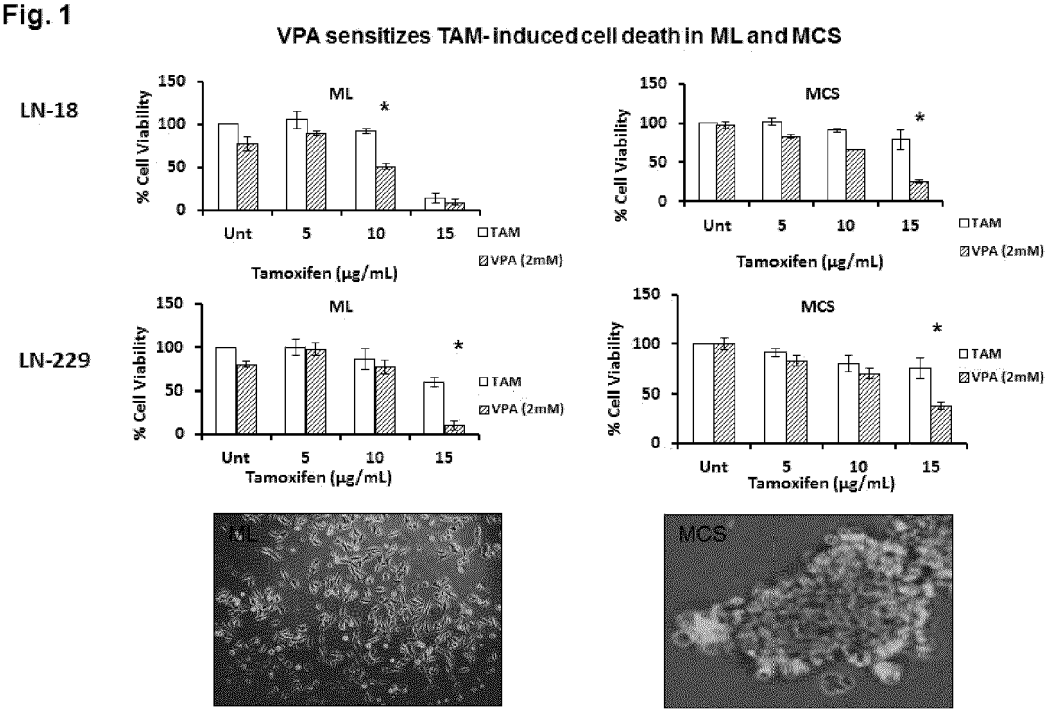
3. La composition pharmaceutique à utiliser selon les revendications 1-2, où les inhibiteurs d'histone-désacétylase (HDACi) sont compris entre 0,5 mM et 5 mM, de préférence entre 1 mM et 5 mM, et de préférence encore entre 1 et 3 mM.

4. La composition pharmaceutique à utiliser selon les revendications 1 à 3, où le tamoxifène est compris entre 1 μg/ml et 20 μg/ml, de préférence entre 5 μg/ml et 15 μg/ml.

5. La composition pharmaceutique à utiliser selon les revendications 1 à 4, où l'effet se situe au niveau de la voie de signalisation RTK/Ras/PI3K, de la voie de signalisation pRB, de la voie PI3K/AKT/mTOR, de la voie p53, de la voie Par-4/GRP78, de préférence de la voie Par-4/GRP78.

6. La composition pharmaceutique à utiliser selon les revendications 1 à 5, où la composition comprend en outre des excipients choisis dans le groupe des conservateurs, des agents tampons, des sels, des supports, des diluants.

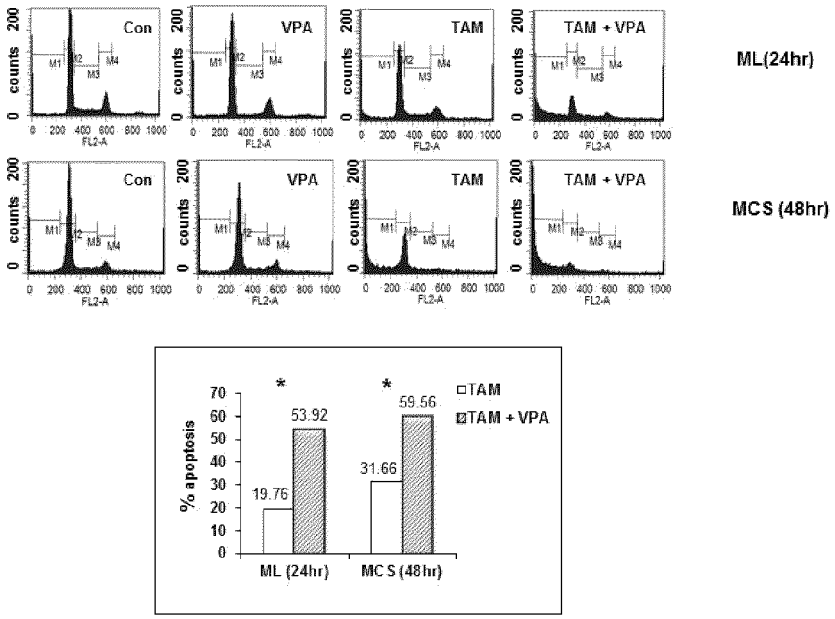
Fig. 1



ML- monolayer, MCS- multicellular spheroids, VPA- valproic acid, % viability calculated considering viability in untreated cells as 100%. * $p<0.05$

FIGURE 1

Fig. 2 VPA sensitizes TAM-induced apoptosis in ML and MCS

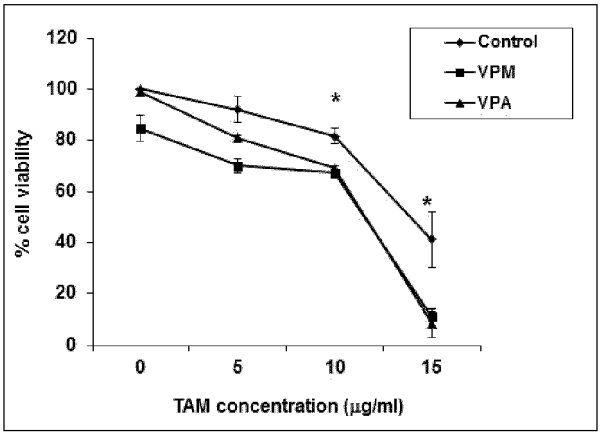


ML- monolayer, MCS- multicellular spheroids, TAM-tamoxifen (10ug/ml), VPA- valproic acid (2mM), * p<0.05

FIGURE 2

Fig. 3

VPM, an analogue of VPA sensitizes TAM- induced cell death in ML in LN-18 cells.



TAM-Tamoxifen (10ug/ml) VPA- valproic acid (2mM), VPM(2mM)
% viability calculated considering viability in untreated cells as 100%. * p<0.05

FIGURE 3

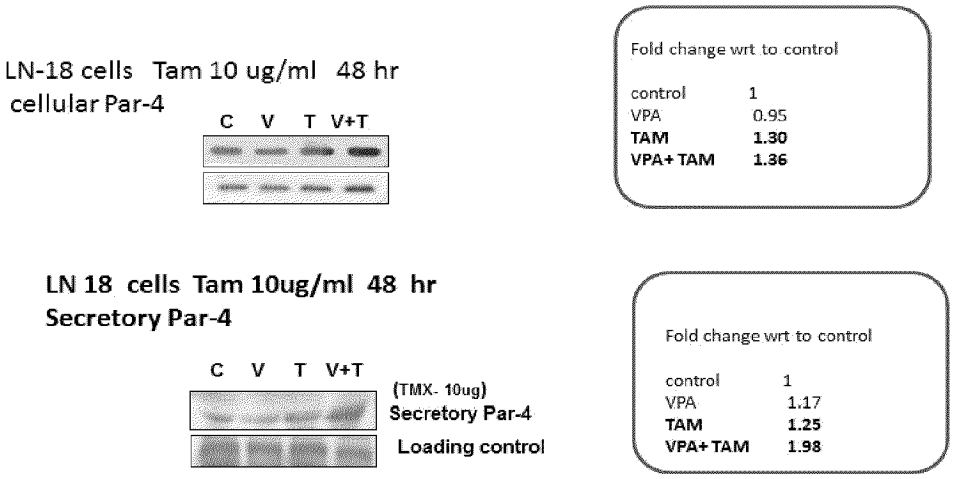

LN-18 cells Tam 10 ug/ml 48 hr
cellular Par-4
C V T V+T
Fold change wrt to control
control 1
VPA 0.95
TAM 1.30
VPA+TAM 1.36
LN 18 cells Tam 10ug/ml 48 hr
Secretory Par-4
C V T V+T
(TMX- 10ug)
Secretory Par-4
Loading control
Fold change wrt to control
control 1
VPA 1.17
TAM 1.25
VPA+TAM 1.98

FIGURE 4

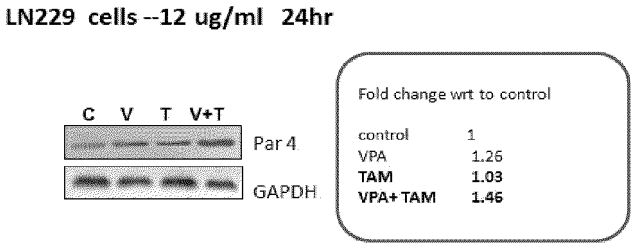

LN229 cells --12 ug/ml 24hr
C V T V+T
Par 4
GAPDH
Fold change wrt to control
control 1
VPA 1.26
TAM 1.03
VPA+TAM 1.46

FIGURE 5

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- US 20090048156 A1 **[0006]**
- US 20080085874 A1 **[0007]**
- WO 2009142968 A **[0009]**
- WO 2008027837 A **[0010]**

Non-patent literature cited in the description

- **P N MUNSTER et al.** A phase II study of the histone deacetylase inhibitor vorinostat combined with tamoxifen for the treatment of patients with hormone therapy-resistant breast cancer. *British Journal Of Cancer, London*, 10 May 2011, vol. 104 (12), 1828-1835 **[0008]**
- **NOEMI ARRIGHETTI et al.** Drug Combinations with HDAC Inhibitors in Antitumor Therapy. *Critical Reviews In Oncogenesis*, 01 January 2015, vol. 20 (1-2), 83-227 **[0011]**